CYTOTAXONOMY OF ILLICIUM FLORIDANUM AND I. PARVIFLORUM (ILLICIACEAE)

DONALD E. STONE AND JUDITH L. FREEMAN [1]

RECENT CHROMOSOME COUNTS of the Ranales by Raven and Kyhos (1965) provide new insight into evolutionary patterns of the most primitive members of extant angiosperms. They concluded that the basic chromosome number for the primitive angiosperms is probably $x=7$. It is argued that numbers such as $n=6$, 8 (Aristolochiaceae), $n=8$, 9 (Annonaceae), $n=10$ (Eupomatiaceae), $n=11$ (Calycanthaceae, Saururaceae), $n=12$ (Lauraceae), $n=13$ (Canellaceae, *Drimys* sect. TASMANNIA), etc. are aneuploid derivatives of the base number 7 and its multiple, $n=14$. Stebbins (1958, 1966) has recently reiterated an alternative hypothesis which seeks to explain the higher numbers such as $n=12$ and $n=13$ as the result of "direct polyploidy," rather than a doubling of 7 followed by aneuploid reduction. He pictures aneuploid changes in chromosome number from a basic $x=6$ or $x=7$ to have gone both upward ($x=9$) and downward ($x=5$) at an early stage of angiosperm evolution. This period was followed by the build up of higher basic numbers in woody members through polyploidy. Once the polyploid levels were reached Stebbins implied that further evolutionary diversification was relegated to processes of speciation and generic differentiation at the homoploid level. While such a hypothesis is highly plausible and, in fact, is generally accepted in the derivation of $n=19$ in the Magnoliaceae from unknown $n=7$ and $n=12$ (found in the related Degeneriaceae and Himantandraceae) ancestors (Stebbins, 1950; Darlington, 1956; Raven and Kyhos, 1965); evidence does exist in the Ranales for aneuploid changes at the polyploid level. The Piperaceae, for example, have $n=11$, 12, 14, and 16, numbers which are surely indicative of aneuploid derivation. Stebbins (1966, *Table 2*) overlooked another example cited by Raven and Kyhos (1965; Stone, 1965) in which *Illicium floridanum* Ellis with $n=13$ is a probable derivative of the basic $n=14$ common to other members of the Illiciaceae and the related Schisandraceae. It is the purpose of this paper to examine the karyotypes of two of the four New World species of *Illicium* and to document the aneuploid reduction from $n=14$ to $n=13$.

MATERIALS AND METHODS

The collections and chromosome numbers of *Illicium floridanum* Ellis and *I. parviflorum* Michx. ex Vent. are presented in TABLE 1. The populations that were sampled do not span the range of each species but presumably are representative. *I. floridanum* ranges from northwestern

[1] Work done in conjunction with research supported by NSF grants GB-2313, GB-5233X, GY-2484, and the Duke University Council on Research. We wish to thank Dr. Carroll E. Wood, Jr. for his helpful comments on the manuscript.

TABLE 1. **Collections and chromosome numbers of Illicium floridanum and I. parviflorum**

SPECIES AND COLLECTION NUMBER	CHROMOSOME NUMBER		COLLECTION DATA
	n	*2n*	
Illicium floridanum Ellis (sect. ILLICIUM)			
Stone 1357	13		Washington Parish, near Warnerton, La. (NO)
Stone 1522		26	Hancock Co., near Logtown, Miss. (DUKE)
Stone 1819		26	Alachua Co., cultivated near Gainesville, Fla., by A. M. Laessle. (DUKE)
Illicium parviflorum Michx. ex Vent. (sect. CYMBOSTEMON)			
Stone 1422	14	28	Alachua Co., cultivated near Gainesville, Fla., by A. M. Laessle. Cuttings originally obtained from Norwalk, ne. corner of Ocala National Forest. (DUKE)
Stone 1820	14	28	Orange Co., Chapel Hill, N.C., cultivated on Univ. of North Carolina campus. (DUKE)
Stone 1821		28	Orange Co., Chapel Hill, N.C., cultivated on Univ. of North Carolina campus. (DUKE)

Florida to eastern Louisiana and northward to central Alabama. *I. parviflorum* is restricted to a few localities at the headwaters of the St. Johns River in northeastern Florida and is quite often marketed by Florida nurserymen as *I. anisatum* (pers. comm., A. M. Laessle).

Flower buds suitable for studies of meiosis were fixed in 3 (absolute ethanol): 1 (glacial acetic acid) and stored in 70% ethanol until prepared for examination by the standard acetocarmine squash technique. Karyotype analyses of mitotic chromosomes were conducted on the root tips of young seedlings or cuttings stimulated to produce callus and adventitious roots with hormone treatment. Most satisfactory preparations were obtained by treating root tips in a 0.1% colchicine solution for 3 to 4 hours before fixation.

Chromosome squashes deemed satisfactory for analysis were photographed with a Leitz Ortholux microscope (× 90 objective, N.A. 1.32) and Kodak Contrast Process Ortho sheet film. Prints of the negatives were standardized at × 3000 for measurement with a millimeter rule. Measurements were made of the total chromosome length and length of each arm. The measurements of homologous chromosomes were averaged

and expressed in percent as a function of the total chromosome length of the genome (i.e. mean percent length, Martin and Hayman, 1965). This procedure was an attempt to reduce variation in chromosome length introduced by variable cell-sizes and squash preparations, while at the same time expressing the results from different cells on a standard scale.

RESULTS

Karyotype analyses. The data from untreated and colchicine treated cells were processed separately. The differences due to treatment were negligible, however, and the samples were subsequently pooled. While the basic proportions of the karyotype were unaffected by treatment, the highly contracted colchicine-treated chromosomes were less suitable than untreated chromosomes for analyses of secondary constrictions. The results presented in TABLE 2 are based on ten cells each from seedlings and cuttings of *Illicium parviflorum* (*Stone 1422, 1820*) and a combined total of eight cells from seedlings and cuttings of *I. floridanum* (*Stone 1522, 1819*).

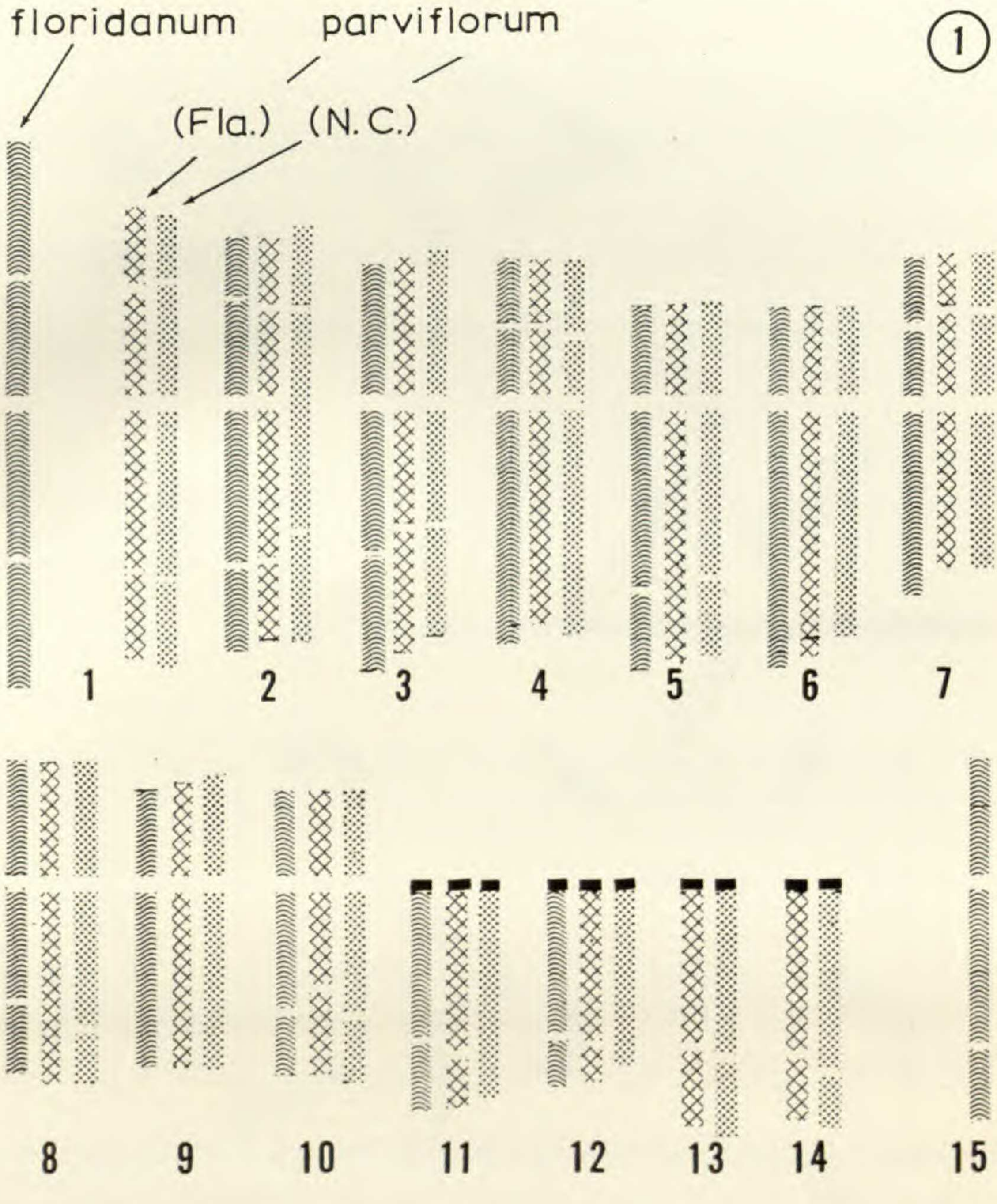
The data from the native Florida (*Stone 1422*) and cultivated North Carolina (*Stone 1820*) plants of *Illicium parviflorum* are presented separately to show the remarkable similarity in karyotypes (FIG. 1). Since these samples were independently analyzed, we consider the good agreement in results to be ample vindication of the techniques employed.

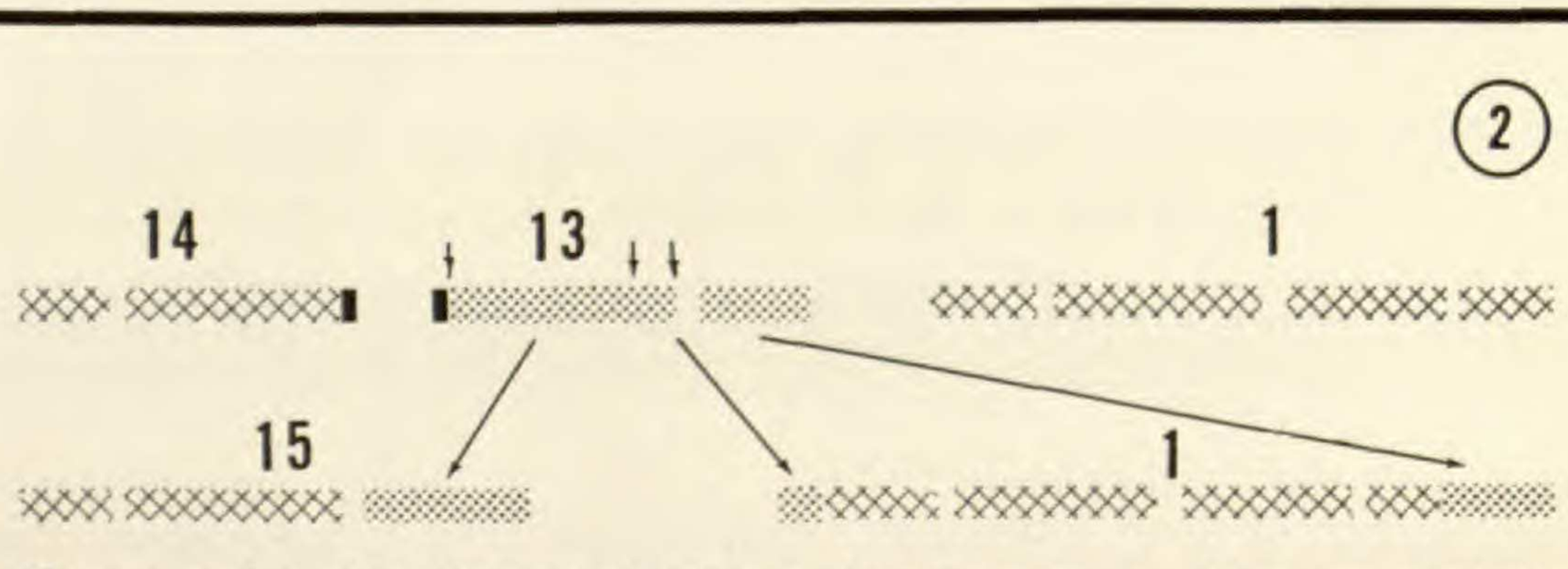
Illicium floridanum has $2n=26$ (FIG. 3) and a karyotype consisting of 1 V-shaped, 10 J-shaped, and 2 rod chromosomes. *I. parviflorum* has $2n=28$ (FIGS. 7, 8) and a karyotype which includes 10 J-shaped and 4 rod chromosomes. On the basis of total size, relative arm lengths, and secondary constrictions, it has been possible to match 9 J (#2–10) and 2 rod chromosomes (#11, 12) of the *I. floridanum* and *I. parviflorum* complements (FIG. 1). Chromosomes 1 and 15 of *I. floridanum* and chromosomes 1, 13, and 14 of *I. parviflorum* do not have identical equivalents. Since *I. floridanum* (sect. ILLICIUM) and *I. parviflorum* (sect. CYMBOSTEMON) are representative of two clearly demarcated sections of the genus, direct derivation of one from the other is not possible. However, if we assume the 14-chromosome karyotype of *I. parviflorum* is probably not too dissimilar from other 14-chromosome species, including *I. anisatum* of sect. ILLICIUM, then the simplest explanation is that three *parviflorum*-like chromosomes have undergone reciprocal translocations of unequal chromosome segments to be repatterned into the two of *I. floridanum*. The basic scheme proposed by Darlington (1937) shows how the entire euchromatic arm of a rod-shaped chromosome could be transferred to the heterochromatic arm of a second rod chromosome. This process, sometimes referred to as centric fusion, followed by loss of one heterochromatic centromere region, would result in gametes with one less chromosome but without any loss of genetic material. Of course, proof for this type of rearrangement must necessarily come from meiotic studies of F_1 species hybrids, as Togby (1943) has demonstrated in *Crepis* ($n = 4$ to $n = 3$) and Kyhos (1965) in *Chaenactis* ($n = 6$ to $n = 5$).

TABLE 2. **Data on the karyotypes of Illicium floridanum and I. parviflorum**
(Measurements expressed in percent as a function of total genome length)

SPECIES	CHROMOSOME LENGTHS	CHROMOSOME NUMBER														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
floridanum	Short Arm	5.99	3.73	3.11	5.22	2.19	4.42	5.16	4.30	4.30	4.78	0.00	0.00	—	—	2.73
	Long Arm	6.31	5.41	5.79	3.22	5.89	3.15	2.25	2.66	2.12	1.85	4.78	5.15	—	—	5.29
	Total Length	12.30	9.14	8.90	8.44	8.08	7.57	7.41	6.96	6.42	6.63	4.78	5.15	—	—	8.02
	Long/Short	1.05	1.45	1.86	1.62	2.68	1.40	2.30	1.62	2.03	2.45	—	—	—	—	1.94
	Absolute Length (μ) *	12.4	9.2	9.0	8.5	8.2	7.5	7.7	7.0	6.5	6.8	5.2	4.8	—	—	8.1
	Chromosome Shape	V	J	J	J	J	J	J	J	J	J	R	R			J
parviflorum (Fla.)	Short Arm	4.21	3.53	3.36	3.19	2.46	2.37	3.43	2.57	2.26	1.92	0.00	0.00	0.00	0.00	—
	Long Arm	5.47	5.20	5.12	4.85	5.61	5.45	3.87	4.89	4.47	4.45	5.11	4.54	5.50	5.36	—
	Total Length	9.68	8.73	8.48	8.04	8.07	7.82	7.32	7.46	6.73	6.37	5.11	4.54	5.50	5.36	—
	Long/Short	1.30	1.47	1.52	1.52	2.28	2.30	1.13	1.90	1.98	2.32	—	—	—	—	—
	Absolute Length (μ) **	10.4	9.4	9.1	8.6	8.7	8.4	7.8	8.0	7.3	6.8	5.5	4.9	5.9	5.8	—
	Chromosome Shape	J	J	J	J	J	J	J	J	J	J	R	R	R	R	—
parviflorum (N.C.)	Short Arm	4.09	3.95	3.43	3.12	2.43	2.30	3.37	2.52	2.35	1.85	0.00	0.00	0.00	0.00	—
	Long Arm	5.56	5.24	4.98	4.91	5.58	5.15	3.81	4.78	4.57	4.50	5.08	4.43	5.89	5.61	—
	Total Length	9.65	9.19	8.41	8.03	8.01	7.45	7.18	7.30	6.92	6.35	5.08	4.43	5.89	5.61	—
	Short/Long	1.36	1.33	1.46	1.57	2.25	2.24	1.13	1.90	1.95	2.43	—	—	—	—	—
	Absolute Length (μ) ***	10.4	9.4	9.1	8.5	8.6	7.7	8.0	7.9	7.4	6.9	5.5	4.8	6.3	6.0	—
	Chromosome Shape	J	J	J	J	J	J	J	J	J	J	R	R	R	R	—

* Average of 8 untreated cells ** Average of 5 untreated cells *** Average of 4 untreated cells





FIGS. 1 and 2. Morphology of **Illicium** chromosomes. 1. Idiogram of karyotype of *I. floridanum* (chromosomes #1–12, 15) and two populations of *I. parviflorum* [Fla. (*Stone 1422*), N.C. (*Stone 1820*); chromosomes # 1–14]. 2. Theoretical derivation of two chromosomes of *I. floridanum* from three *parviflorum*-like chromosomes by reciprocal translocations of unequal chromosomal segments.

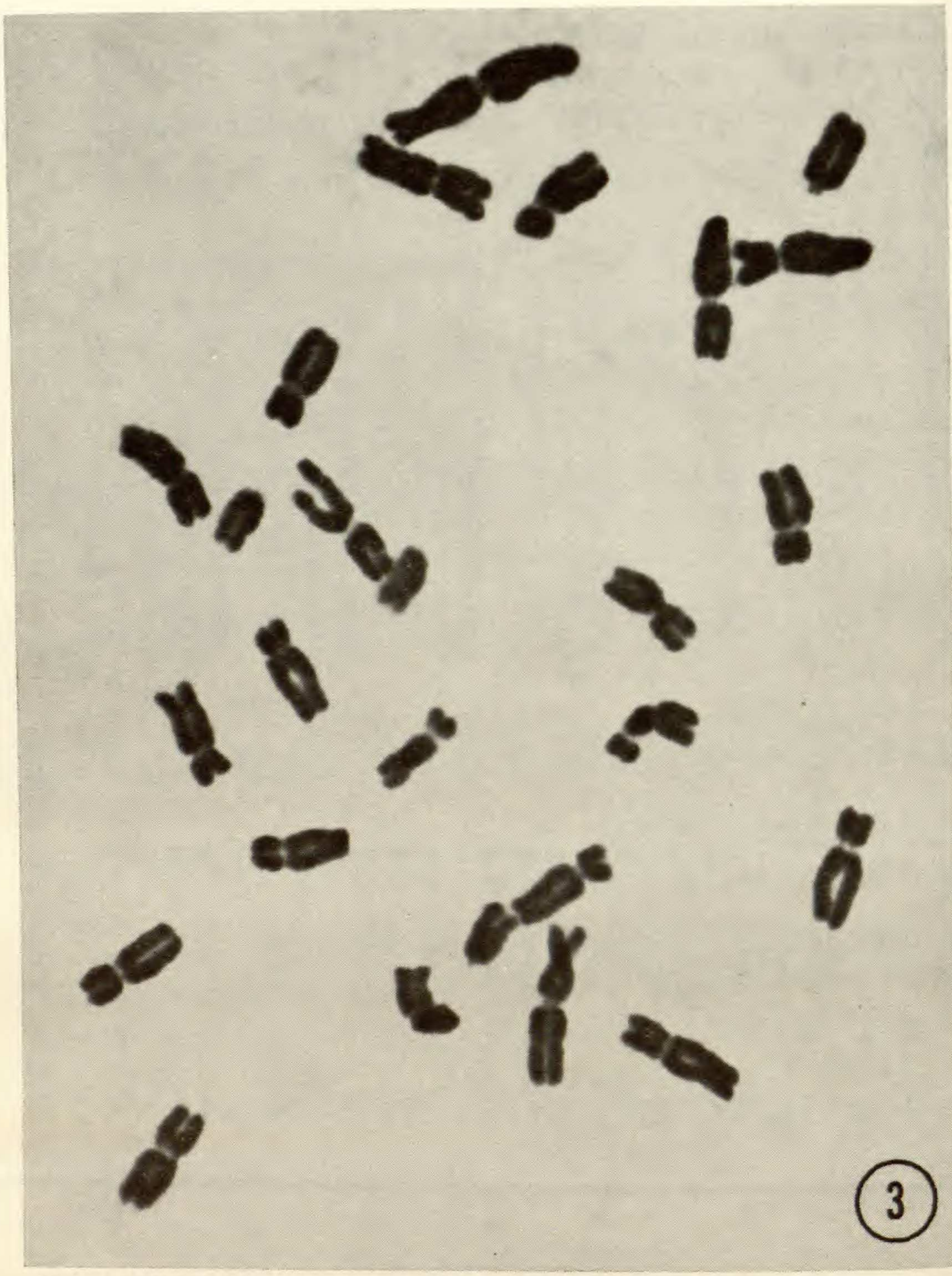


FIG. 3. **Illicium floridanum.** Mitotic metaphase, $2n = 26$, *Stone 1522*, × 1780.

Neither hybrids between these shrubs nor studies of pairing configurations in meiosis have been attempted. However, the simplest explanation for the derivation of $n=13$ from $n=14$ would involve centric fusion. Fusion of rod chromosomes 13 and 14 (FIG. 2) might have produced chromosome 15 of *I. floridanum*. Chromosome 1 of *I. floridanum* (FIG. 1) is very similar to chromosome 1 of *I. parviflorum*. Both chromosomes are the longest of their respective genomes and both have secondary constrictions located in approximately the same region in each arm. The

J-shaped morphology of *I. parviflorum* could have been transformed into the v-shape of *I. floridanum* by adding segments to the ends of the arms (FIG. 2).

Meiotic analyses. Acetocarmine squashes of pollen mother cells were prepared. Meiosis was found to be normal in both species with a base number of $n=13$ in *Illicium floridanum* (FIGS. 4–6) and $n=14$ in *I. parviflorum* (FIGS. 9–10). An analysis of 19 cells of *I. floridanum* revealed an average chiasma-frequency of 1.31 per bivalent (TABLE 3).

TABLE 3. **Chiasma-frequency at diakinesis in Illicium floridanum** *

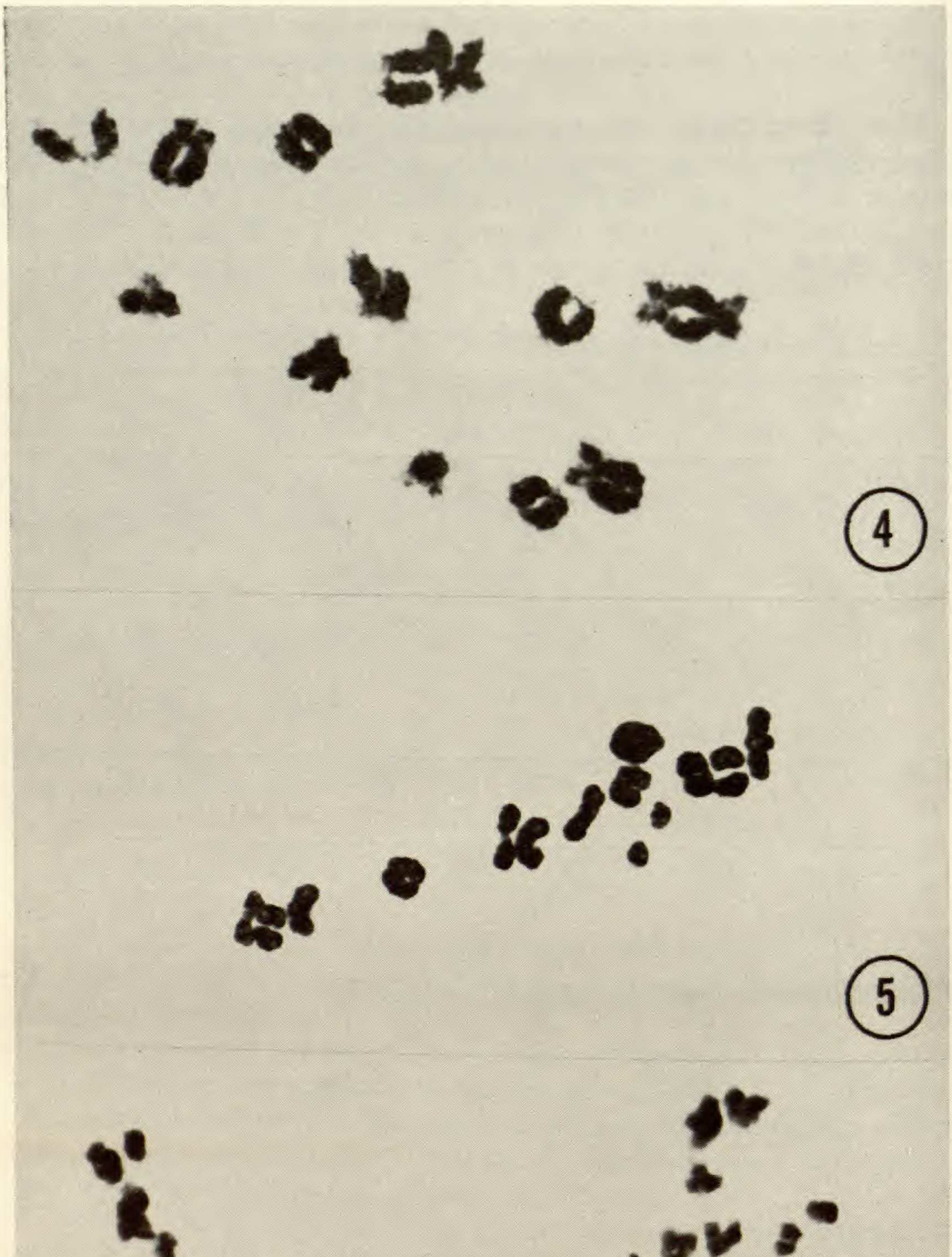
TYPES OF CHIASMATA	NO. OF CHROMOSOMES	NO. OF CHIASMATA	% CHIASMA-TYPES
Zero	16	0	6.5
One terminal	69	69	27.9
Two terminals	35	70	14.2
One interstitial	73	73	29.6
Two interstitials	25	50	10.1
One terminal — one interstitial	4	12	1.6
TOTALS	247	324	100.0
Chiasma-frequency/bivalent = 324/247 = 1.31			

* Based on 19 cells

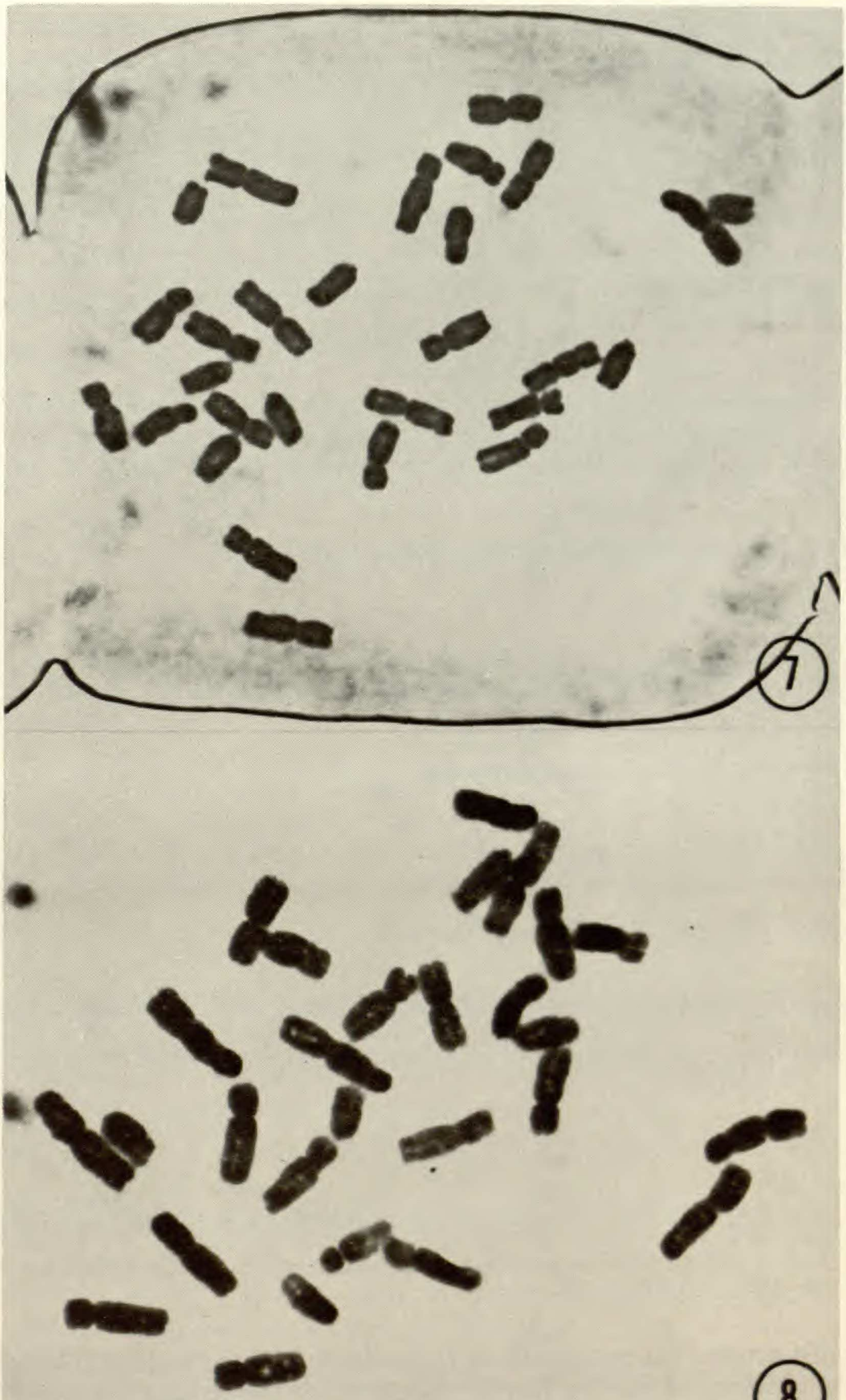
SUMMARY AND CONCLUSIONS

Chromosome counts on the related Schisandraceae (Whitaker, 1933; Stone, 1965) and two of the three members of the Illiciaceae (Whitaker, 1933), suggest that 14 is the base number for the alliance. Viewed in this light, $n=13$ in *Illicium floridanum* clearly seems to be the product of aneuploid reduction. Though we have made direct comparisons between the karyotypes of *I. floridanum* (sect. ILLICIUM) and *I. parviflorum* (sect. CYMBOSTEMON), it should be re-emphasized that these species are representatives of the two sections of the family. It would be most interesting to examine the 14-chromosome karyotype of *I. anisatum* (Whitaker, 1933), for this species is one of the twelve Smith placed in section ILLICIUM (formerly sect. BADIANA) with *I. floridanum*. The cytology of section CYMBOSTEMON is equally unknown. The $n=14$ count of *I. parviflorum* is the only report on the 29 species recognized by Smith (1947). While it is quite likely that the 14-chromosome species of both sections have similar karyotypes, it will be necessary to analyze close relatives of *I. floridanum* and meiotic pairing configurations formed in $n=13 \times n = 14$ hybrids before the exact nature of aneuploid reduction is established.

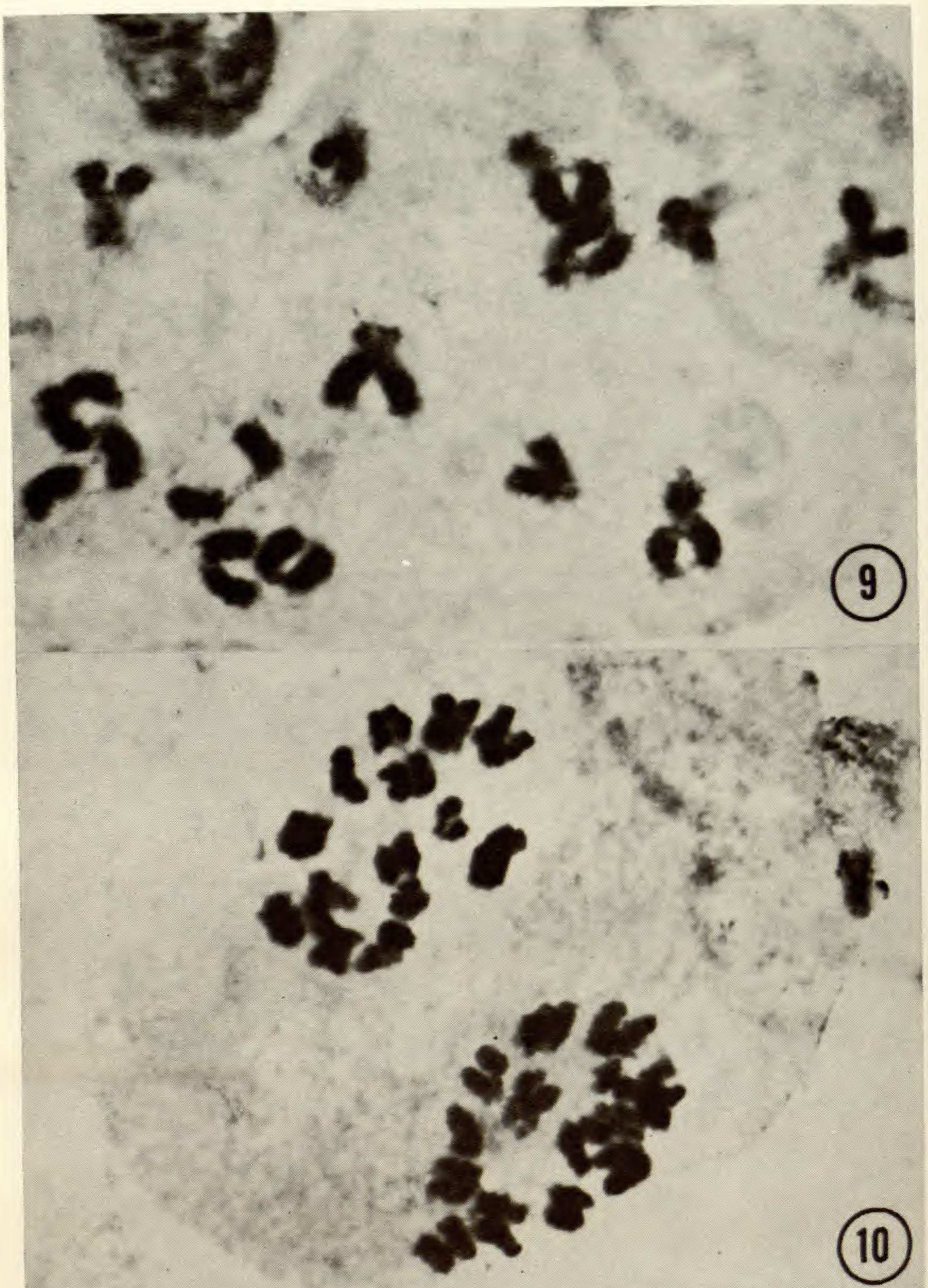
Centric fusion (FIG. 2) of rod chromosomes 13 and 14 of *I. parviflorum*



FIGS. 4–6. **Illicium floridanum.** Meiosis in pollen mother cells, $n = 13$, *Stone 1357*, × 1000. 4. Diakinesis. 5. Metaphase I. 6. Anaphase I.



FIGS. 7 and 8. **Illicium parviflorum.** Mitotic metaphase, $2n = 28$, × 1780. 7. North Carolina population, *Stone 1820*. 8. Florida population, *Stone 1422*.



FIGS. 9 and 10. **Illicium parviflorum.** Meiosis in pollen mother cells, $n = 14$, *Stone 1820*, × 1500. 9. Diakinesis. 10. Anaphase I.

could account for number 15 of *I. floridanum* (FIG. 1). In addition, if the residuum of number 13 was transferred to the terminal ends of the arms of chromosome 1 (FIG. 2), a v-shaped chromosome similar to chromosome 1 of *I. floridanum* would result.

We have no information on the significance of the reduction from $n=14$ to $n=13$. Whitaker (1933) originally reported $n=14$ for *Illicium anisatum* L. (as *I. religiosum*) and *I. floridanum*. As we have shown, his report for *I. floridanum* is probably incorrect. Unfortunately, as Wood (1958) has noted, Whitaker's counts were not documented with voucher specimens and there is no way to tell if he miscounted or misidentified the material. It does open the question, however, of whether *I. anisatum* of the same section might not also have $n=13$. If not, then $n=13$ may be exclusive to the American species of section ILLICIUM. For as Smith (1947) commented, "The species [*I. floridanum*] is clearly separable from all Old World members of § *Badiana* [= § ILLICIUM] on the basis of its comparatively long pedicels, numerous stamens, and brightly colored perianth segments. Its only close relative is the following new species [*I. mexicanum*]."

LITERATURE CITED

BAILEY, I. W., & C. G. NAST. 1948. Morphology and relationships of *Illicium*, *Schisandra* and *Kadsura*. I. Stem and leaf. Jour. Arnold Arb. **29**: 77–89.

DARLINGTON, C. D. 1937. Recent advances in cytology. 2 ed. Blakiston, Philadelphia.

———. 1956. Chromosome botany. The University Press, Aberdeen.

KYHOS, D. W. 1965. The independent aneuploid origin of two species of *Chaenactis* (Compositae) from a common ancestor. Evolution **19**: 26–43.

MARTIN, P. G., & D. L. HAYMAN. 1965. A quantitative method for comparing the karyotypes of related species. Evolution **19**: 157–161.

RAVEN, P. H., & D. W. KYHOS. 1965. New evidence concerning the original basic chromosome number of angiosperms. Evolution **19**: 244–248.

SMITH, A. C. 1947. The families Illiciaceae and Schisandraceae. Sargentia **7**: 1–224.

STEBBINS, G. L., Jr. 1950. Variation and evolution in plants. Columbia University Press, New York.

———. 1958. On the hybrid origin of angiosperms. Evolution **12**: 267–270.

———. 1966. Chromosomal variation and evolution. Science **152**: 1463–1469.

STONE, D. E. 1965. *In*: Documented chromosome numbers of plants. Madroño **18**: 125.

———. In press. Cytological and morphological notes on the southeastern endemic, *Schisandra glabra* (Schisandraceae). Jour. Elisha Mitchell Soc.

TOGBY, H. A. 1943. A cytological study of *Crepis fuliginosa, C. neglecta*, and their F_1 hybrid, and its bearing on the mechanism of phylogenetic reduction in chromosome number. Jour. Genet. **45**: 67–111.

WHITAKER, T. W. 1933. Chromosome number and relationship in the Magnoliales. Jour. Arnold Arb. **14**: 376–385.

WOOD, C. E., Jr. 1958. The genera of the woody Ranales in the southeastern United States. Jour. Arnold Arb. **39**: 315–318.

DEPARTMENT OF BOTANY
DUKE UNIVERSITY
DURHAM, NORTH CAROLINA 27706